

123 A shotgun metaproteomics approach to study the faecal microbiome of patients with cystic fibrosis reveals a reduction of butyrate-producing bacteria

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Objectives: The gut microbiome is a diverse and complex microbial ecosystem. In patients with cystic fibrosis (CF) this microbiome is influenced due to antibiotic treatments, the enlarged mucus layer in the small intestine, the changed pH due to the decreased bicarbonate transport in the intestinal lumen and the different alimentary environment due to decreased release of digestive enzymes by the pancreatic duct. These problems not only entail changes in the composition of the gut microbiome, but also cause intestinal inflammation and affect the general well-being of CF patients.

Methods: A shotgun metaproteomics approach is used to characterize and compare the predominant members of the faecal microbiome. Faecal protein extracts obtained from seven CF patients and their siblings were separated by SDS-PAGE and in-gel digested. The tryptic peptides were analyzed using LC-ESI FT-ICR-MS. The detected peptide sequences were analyzed using UniPept (<http://unipept.ugent.be>) and Progenesis LC-MS was used to determine the differentially expressed proteins.

Conclusion: UniPept analysis shows that bacteria of the phylum Firmicutes, and in particular several butyrate-producing bacteria were underrepresented in CF patients. Butyrate serves as the preferred energy source for the colonocytes, has anti-inflammatory effects and therefore aids in the improvement of the intestinal barrier. Furthermore the differentially expressed protein study shows that four proteins of the butyrate synthesis pathway (acetyl-CoA acetyltransferase, β -hydroxybutyryl-CoA dehydrogenase, 3-hydroxybutyryl-CoA dehydratase and butyryl-CoA dehydrogenase) are less abundant in the faecal microbiome of CF patients.

124 Ribosomal RNA intergenic spacer analysis (RISA) as a rapid and diagnostic means to profile microbial diversity in cystic fibrosis sputum

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Objectives: Cultivation-independent analysis has revealed the polymicrobial nature of CF lung infection. The literature increasingly suggests that low sputum microbial diversity is associated with poor lung function in CF. The optimal means of profiling bacterial diversity in routine CF care is unknown. We assessed the suitability of a simple PCR-profiling tool, Ribosomal Intergenic Spacer Analysis (RISA), for the investigation of sputum bacterial diversity.

Methods: Paired sputum samples were sent for routine microbiology and subjected to total nucleic acids extraction using an automated method applied in standard clinical virology. Microfluidics was used to separate RISA profiles amplified from this DNA. 16S rRNA gene pyrosequencing was performed on a subset of samples to evaluate the performance of RISA.

Results: RISA profiles were successfully amplified from 200 sputum samples representative of 93 CF patients. 25% of these patients demonstrated RISA profiles dominated by one of the following Gram negative bacteria: *Burkholderia cepacia* complex, *Ralstonia mannitolilytica*, *Stenotrophomonas maltophilia* and *Achromobacter xylosoxidans*. Pyrosequencing analysis confirmed the loss of microbial diversity in these samples. In 9 cases conventional microbiology failed to correctly identify these dominant bacterial pathogens. The RISA profiles of UK CF patients infected with the *B. cenocepacia* ET12 strain matched those of Czech patients infected with the ST32 strain.

Conclusions: Infection with non-fermentative Gram negative bacterial species leads to a loss in microbial diversity in CF that can be rapidly detected using a simple RISA profiling method.

125 Investigation of cystic fibrosis airway microbiome in patients showing a severe decline in lung function and not responding to conventional antimicrobial therapy

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Objectives: The objectives of the present project are to assess the composition of airways microbiota in CF patients with a severe decline in lung function and not responding to antimicrobial therapy, and to discover new opportunistic (non-culturable) pathogens involved in pulmonary disease. Targeted antibiotic therapy against other members of the polymicrobial community can contribute to resolve pulmonary disease in CF patients.

Methods: Three groups of CF patients were investigated:

1. normal lung function/mild decline (FEV₁, >70% of predicted),
2. moderate lung dysfunction (FEV₁, 40 to 69% of predicted) and
3. severe lung dysfunction (FEV₁ <40% predicted).

Within each group, "non-responder" CF patients who have showed a severe decline in lung function (FEV₁ \geq 5% in the last year) and did not respond to antimicrobial therapy, and "stable CF patients" (having had no change in pulmonary function or a rate decline in FEV₁ equal to average value in the last year) has been enrolled. Composition of CF microbiota was investigated by using advanced culture-based methods and the molecular profiling method of Terminal Restriction Fragment Length Polymorphism (T-RFLP).

Conclusion: Our preliminary findings suggest that combining culture-dependent and culture-independent approaches provides a more comprehensive perspective of CF microbiology than either approach alone. The results obtained will set the basis to identify new targets for treatment and management of bacterial infections in CF patients.

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126 Changes in the microbiology of the pulmonary secretions in cystic fibrosis (CF) patients: Report on 4 different years during 25 years

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Our laboratory has been studying respiratory secretions of CF since 1987 and during these 25 years lots of changes were observed.

The aim of this study was to analyse microbiological findings in 4 different years between 1987 and 2011.

Sputum, oropharyngeal swab and BAL from 329 pts, aged 0.1–20 yo, collected in 1987, 1995, 2005 and 2011 were analysed. Samples were plated on 5% blood agar, chocolate agar, CLED agar and mannitol salt agar. Since 2001 BCSA agar was used, to enhance *B. cepacia* recovery.

The number of patients (cultures) in each year was 70 (108), 82 (217), 124 (384) and 120 (498) in 1987, 1995, 2005 and 2011, respectively. The median (range) of culture/patient was 1 (1–4), 2 (1–9), 3 (1–9) and 4 (1–11) in each year.

The percentages of patients' isolates are presented in the table.

Percentages of patients' isolates

	1987	1995	2005	2011	p (1987 vs. 2011)
<i>P. aeruginosa</i>	76.6	72.8	58.6	44.4	<0.01
<i>S. aureus</i>	51.9	78.3	79.7	71.8	<0.01
MRSA	5.2	22.8	33.6	32.3	<0.01
<i>H. influenzae</i>	10.4	19.6	26.6	41.9	<0.01
<i>B. cepacia</i>	2.6	1.1	18.7	6.5	ns
<i>A. xylosoxidans</i>	0	0	4.7	4.8	ns
<i>S. maltophilia</i>	0	2.2	3.1	8.9	ns

Conclusions: A decrease in *P. aeruginosa* colonisation was observed. An increasing rate of *S. aureus* and MRSA colonisation was noted. A peak of *B. cepacia* colonisation was detected in 2005.